Attorney Docket: UCONBA/186/US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: Ben A. BAHR

Application No.:	10/056,666	Examiner:	Michael V. MELLER
Filing Date:	10/29/2001	Group Art Unit:	1654

For: Materials For Lysosome Modulation And Methods Of Use Thereof

Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. 1.131

I, Ben A. Bahr, hereby declare:

1. I am the inventor named in the above-identified U.S. patent application.

2. I am familiar with the Office communications mailed on 5/6/2003 and 10/20/2003 in the above-identified application and with International Publication Number WO 00/56335 cited therein having an International Publication date of September 28, 2000.

3. Attached hereto are documents containing facts showing a completion of my invention in this country before the September 28, 2000 International Publication Date of International Publication Number WO 00/56335. Some dates and information unrelated to the date of invention on these documents have been redacted. The redacted dates are prior to September 28, 2000.

4. Exhibit A is a copy of a University Of Connecticut Invention Disclosure (Disclosure No. 00-004) containing facts showing a completion of my invention in this country. The invention disclosure was signed by me on December 30, 1999 and was witnessed on January 10, 2000.

5. Exhibit B is a copy of a Non-Confidential Disclosure, UConn Case No: 00-004. This Non-Confidential Disclosure refers to the University Of Connecticut Invention Disclosure of Exhibit A (Disclosure No. 00-004) and was prepared prior to September 28, 2000.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

by: _____
Ben A. Bahr

date: January 15, 2004

Exhibit A

JAN 2000
Center for Science & Technology Commercialization Received

THE UNIVERSITY OF CONNECTICUT
INVENTION DISCLOSURE

TECHNOLOGY TRANSFER OFFICE
RESEARCH FOUNDATION

Disclosure No. 00-004
(Leave Blank)

Inventor(s) Full Name	Department/Campus	Office Phone	Home Street Address
Revenue Share: %	Office Address/U-Box	Citizenship	City, State, Zip Code
1. Ben A. Bahr	Pharmaceutical Sci	860-486-6043	100 Grant Ave
100 %	Storrs Campus,U-92 CT 06269-2092	U.S.	Stafford Springs,CT 06076
2.			
%			
3.			
%			

TECHNICAL TITLE OF INVENTION:
Cellular Regulation to Suppress Neuropathogenesis
LAYMAN'S TITLE OF INVENTION:
Treatment for the Pathogenic Processes of Neurodegeneration
FINANCIAL SUPPORT: Describe the funding source(s) for the work that led to this invention.

	Source	Grant/Contract No.	Indicate the major source (ONE ONLY)
University			
Government Agency			

INVENTOR AND WITNESS SIGNATURES: By signing, the INVENTOR(S) confirm: (1) The completeness and accuracy of the information in this disclosure; (2) Their willingness to cooperate fully with the Technology Transfer Office during the evaluation, patenting (if appropriate), and commercial development of this invention; and (3) Their agreement to share the inventors' portion of any revenues derived from this invention according to the percentages given above on this page. By signing, the WITNESS(ES), who cannot be co-inventors, confirm that they have read and understood the invention disclosure as of the signing date.

Inventor(s) 1. Ben A. Bahr Date 12/30/99 Disclosed to and Understood by:

2. _____ Date _____ Jeremy Bender Date 1-10-00

3. _____ Date _____ Queenie Brown Date 1-10-00

INVENTION DISCLOSURE PAGE 2 Disclosure No._____

(1) COMPLETE DESCRIPTION OF THE INVENTION: Use additional pages, if necessary, and attach any relevant sketches, diagrams, drawings, photographs or other illustrative material. ALL ATTACHED MATERIALS MUST BE SIGNED AND DATED BY EACH INVENTOR AND WITNESSED. Description may be by reference to a separate document such as a publication, manuscript, preprint or report. Such documents must be attached.

Lysosomal disturbances are known to induce many types of neurodegenerative processes including those associated with Alzheimer's disease. Using a model system, I have found compelling evidence in support of a specific pathogenic cascade that is initiated by lysosomal dysfunction and leads to the types of synaptic deterioration linked to cognitive decline and dementia. Indeed, many of the pathogenic steps identified in the model were evident in correlative analyses using tissue samples from Alzheimer patients, the more prominent events being abnormal processing and accumulation of proteins and protein fragments. The present invention describes the modulation of cathepsin enzymes *in vitro* and *in vivo* to cause their up-regulation and, concomitantly, to enhance lysosomal capacity in neurons and other cell types (see Supplement pages). Associated with the increased lysosomal capacity is a dramatic decline in abnormal protein processing. Such regulation of cellular functions will offset those accumulations of aberrant proteins found associated with various pathologies and, thus, provide a treatment to delay or slow neurodegenerative events including those underlying Alzheimer's disease, Parkinson's disease, and lysosomal storage disorders.

(2) NOVEL FEATURES: Clearly specify the novel aspects of your invention. Compared to present technology, how is your invention different?

The invention targets the broad action of cellular functions related to lysosomes.

What deficiency in the present technology does your invention improve upon? Is it more effective? cheaper? superior in other ways?

(3) STAGE OF DEVELOPMENT: Cite your specific results to date demonstrating that your concept is valid. Has your work included laboratory studies? Pilot-scale experiments? Construction and testing of a prototype?

To validate the concept, laboratory studies with an *in vitro* model of Alzheimer's disease demonstrated that lysosomal capacity can be up-regulated in such a way as to attenuate the production of abnormally processed proteins. Pilot *in vivo* studies also confirmatory.

Inventor(s) 1. Ian A. Yankner Date 12/30/99 Disclosed to and Understood by:

2. _____ Date_____ Lorna Brandt Date 1-10-00

3. _____ Date_____ Deanna Braun Date 1-10-00

(4) VARIATIONS OF THE INVENTION: Discuss all alternate forms that you can foresee for this invention, whether or not you have evaluated them to date. (For example, chemical inventions should consider analogs and derivatives.)

Alternatives include the use of any compound or agent that can modulate lysosomal function.

(5) DOCUMENTATION OF THE INVENTION: On what date was the invention first conceived? Please identify laboratory notebooks and other written materials that document this date and subsequent dates on which the concept was proven or a prototype was developed.

in notebooks Bahr-J113, MS-72, and MS-77.

(6) INVENTOR'S PUBLICATION PLANS: Please list all your publications — theses, reports, pre-prints, abstracts, papers, etc. that pertain to the invention. Include publication dates. Also, include manuscripts for publication (submitted or not), news releases, and internal publications. Enclose copies of all the above items with this disclosure.

No publications to date.

(7) PRIOR DISCLOSURE: Please give the details (date, place and circumstances) of any oral or written disclosures of all or part of this invention. If disclosed to specific individuals, give their names. Include professional meetings and conferences. Has this invention or a product resulting from this invention been offered for sale or license? Have any samples related to this invention been distributed?

Inventor(s) 1. R. A. Bahr Date 12/30/99 Disclosed to and Understood by:

2. _____ Date _____ Jeremy Brown Date 1-10-00

3. _____ Date _____ Queenie Brown Date 1-10-00

SUPPORTING INFORMATION

(1) PRIOR KNOWLEDGE AND COMPETING RESEARCH AND DEVELOPMENT: Please list all publications and patents by the inventor or others that relate to the invention. The inventor should thoroughly search the published literature and review closely related patents.

List any known research groups currently engaged in research and development in this area. Include both academic and industrial researchers. Ralph Nixon

(2) ALTERNATE TECHNOLOGY: Describe any known alternate technologies that accomplish the same or similar purposes as this invention. List companies and products that currently use these alternate technologies.

(3) COMMERCIAL APPLICATION OF THE INVENTION: List all products, processes, devices, equipment, etc., to which your invention could be applied or which could result directly from your invention. Can these applications be developed in the near term (within two years) or the long term (more than two years)?

pharmaceutical agents for Alzheimer's disease, etc. (long-term).

What firms or types of firms do you think may be interested in the invention? Why? Name companies and specific persons if possible. Especially list companies with which you have had direct contact.

Pfizer

(4) RESEARCH AND DEVELOPMENT PLANS: What additional research is needed to complete development and testing of the invention? Are you actively pursuing the needed work? Under whose sponsorship? About how long will this work take? What additional research support, if any, is needed for these efforts?

Further testing for protection in Alzheimer model at UCSNN.

(5) DISADVANTAGES OF THE INVENTION: From the perspective of the "Devil's Advocate", what is the greatest obstacle to the commercial adoption of your invention? What other weaknesses and disadvantages does it have?

SUPPLEMENT PAGE

INVENTION DISCLOSURE FORM

Disclosure No. _____

The present invention, thus far, has been developed in a model system. The *in vitro* model, an alternative to typical Alzheimers studies, utilizes a system that can maintain slices of rat brain tissue in culture for months. For the model, the hippocampus is used since age-related synaptic changes and Alzheimer-type pathogenesis are concentrated there. Hippocampal slice cultures exhibit many key features of the adult brain including native circuitry, cellular organization, synaptic density, and memory-related plasticity. When subjected to a variety of insults the cultures express a pathologic responsiveness which, particularly at the synapse level, is similar to that expected from *in vivo* studies. Moreover, the model's pathogenic responses to age-related events are similar in sensitivity and temporal relationship to those found in the aged human brain and Alzheimer's disease. It is concluded that cultured slices provide a valuable approach for studying those signal transduction events associated with age-related synaptic dysfunction, doing so without the complexities stemming from systemic variables.

With the slice model, I found evidence supporting a cascade that is initiated by gradual, age-related disturbances of lysosomal activity. The lysosomotropic conditions are followed by 1) modification of tubulin chemistries, 2) hyperphosphorylation and aggregation of the microtubule-associated protein tau, 3) concomitant destabilization of microtubules, 4) disruption of axonal and dendritic transport processes, 5) reduction in presynaptic components and, thus, maintenance, and 6) corresponding deterioration of postsynaptic structures and their electrical responses. This cascade likely leads to the type of cognitive decline associated with aging and in early Alzheimers; as many of the pathogenic steps were evident in correlative analyses using tissue samples from Alzheimer patients.

Lysosomal Modulation and the Reduction of NFT Components. In order to test if lysosomal capacity can be augmented in such a way as to prevent abnormal protein processing, I identified lysosomal modulators that stimulate cellular feedback processes for the up-regulation of hydrolase levels. Low concentrations of Phe-Ala-diazomethylketone exhibited such lysosomal modulation. The diazomethylketone derivative markedly up-regulated cathepsin D, the same hydrolase involved in the compensatory response to Alzheimer-type pathogenesis, at concentrations that caused no detrimental effects to synapses. Lysosomal modulation also was expressed by Phe-Phe-diazomethylketone and Glycinyl-L-phenylalanyl-glycine-semicarbazone. From the group of 20 different compounds tested, the diazomethylketone family contains the best lysosomal modulators.

Inventor(s) 1. _____ Date 12/30/99 Disclosed to and Understood by:

2. _____ Date _____ _____ Date 1-10-00

3. _____ Date _____ _____ Date 1-10-00

SUPPLEMENT PAGE

INVENTION DISCLOSURE FORM

Disclosure No. _____

In laboratory experiments, Phe-Ala-diazomethylketone was applied to cultured slices at a low concentration (10 μM) in order to determine if the up-regulated levels of cathepsin D could be sustained on a long-term basis. It was determined that cathepsin D could be stably enhanced over a 20 day period without any reduction in synaptic markers. The diazomethylketone administered orally to rats also caused similar up-regulation of the cathepsin. More importantly, as the cathepsin hydrolase was modulated in slices over the extended period, hyperphosphorylated species of the microtubule-associated protein tau were gradually reduced in concentration. This suggests that lysosomal modulation is one way in which to dissipate pathogenic precursors of neurofibrillary tangles and prevent synaptic deterioration. Interestingly, a gradual increase in non-pathogenic tau isoforms also corresponded with the changes in cathepsin D and hyperphosphorylated tau levels. Thus, while purified cathepsin D has been shown to degrade tau proteins, lysosomal modulation via the diazomethylketone may also be influencing phosphatases that convert pathogenic tau species into smaller non-pathogenic proteins. The normal tau molecules would then be more readily available to help stabilize microtubules and other transport machinery important for neuronal functions. Such a correlation between the two forms of tau was also evident in the hippocampus of aged mice, where the change vs. age relationships determined for hyperphosphorylated and non-phosphorylated tau were of equal but opposite slopes (Bahr and Vicente, 1998, *J. Neuropathol. Exp. Neurol.* 57:111-121). Thus, along with the fact that lysosomal perturbations do not exhibit irreversible features (Bahr *et al.*, 1994, *Exp. Neurol.* 129:81-94), therapeutic strategies targeting lysosomal capacity appear not to be up against a "runaway train" syndrome. Lysosomal modulators may thereby intervene in the progression of certain types of neuronal atrophy, as well as in the cellular processes that contribute to synaptic loss and the severity of Alzheimers-type dementia.

Inventor(s) 1. _____ Date 12/30/99 Disclosed to and Understood by:

2. _____ Date _____ _____ Date 1-10-00

3. _____ Date _____ _____ Date 1-10-00

Exhibit B

NCD

Lysosomal disturbances are known to induce many types of neurodegenerative processes including those associated with Alzheimer's disease. Using a model with pathogenic responses similar in sensitivity and temporal relationship to those found in Alzheimer's disease, I have found compelling evidence in support of a specific pathogenic cascade that is initiated by lysosomal dysfunction. The lysosomotropic conditions are followed by 1) modification of tubulin chemistries, 2) hyperphosphorylation and aggregation of the microtubule-associated protein tau, 3) concomitant destabilization of microtubules, 4) disruption of axonal and dendritic transport processes, 5) reduction in presynaptic components and, thus, maintenance, and 6) corresponding deterioration of postsynaptic structures and their electrical responses. This cascade likely leads to the types of synaptic deterioration linked to cognitive decline and dementia. Indeed, many of the pathogenic steps identified in the model were evident in correlative analyses using tissue samples from Alzheimer patients, the more prominent events being abnormal processing and accumulation of proteins and protein fragments. The present invention describes the modulation of cathepsin enzymes *in vitro* and *in vivo* to cause their up-regulation and, concomitantly, to enhance lysosomal capacity in neurons and other cell types. Such regulation of cellular functions will offset those accumulations of aberrant proteins found associated with various pathologies and, thus, provide a treatment to delay or slow neurodegenerative events including those underlying Alzheimer's disease, Parkinson's disease, and lysosomal storage disorders

In order to test if lysosomal capacity can be augmented in such a way as to prevent abnormal protein processing, I identified lysosomal modulators that stimulate cellular feedback processes for the up-regulation of hydrolase levels. The modulators were applied to cultured slices and found to cause marked up-regulation of cathepsin D, the same hydrolase involved in the compensatory response in Alzheimer's disease. It was determined that cathepsin D could be stably enhanced over a 20 day period without any reduction in synaptic markers. Oral administration to rats also caused similar up-regulation of the cathepsin. More importantly, as the cathepsin hydrolase was modulated in slices over the extended period, hyperphosphorylated species of the microtubule-associated protein tau were gradually reduced in concentration. This suggests that lysosomal modulation is one way in which to dissipate pathogenic precursors of neurofibrillary tangles and prevent synaptic deterioration. Interestingly, a gradual increase in non-pathogenic tau isoforms also corresponded with the changes in cathepsin D and hyperphosphorylated tau levels. Thus, while purified cathepsin D has been shown to degrade tau proteins, lysosomal modulation may also be influencing phosphatases that convert pathogenic tau species into smaller non-pathogenic proteins. The normal tau molecules would then be more readily available to help stabilize microtubules and other transport machinery important for neuronal functions. Such a correlation between the two forms of tau was also evident in the hippocampus of aged mice, where the change vs. age relationships determined for hyperphosphorylated and non-phosphorylated tau were of equal but opposite slopes (Bahr and Vicente, 1998, *J. Neuropathol. Exp. Neurol.* 57:111-121). Thus, along with the fact that lysosomal perturbations do not exhibit irreversible features (Bahr *et al.*, 1994, *Exp. Neurol.* 129:81-94), therapeutic strategies targeting lysosomal capacity appear not to be up against a "runaway train" syndrome. Lysosomal modulators may thereby intervene in the progression of certain types of neuronal atrophy, as well as in the cellular processes that contribute to synaptic loss and the severity of Alzheimers-type dementia.

Model for studying lysosomal enzymes, and its relationship to Alzheimer's Disease.

Non-Confidential Disclosure, UConn Case No: 00X-004

The present invention describes a model for studying the modulation of cathepsin enzymes *in vitro* and *in vivo* to cause their up-regulation and, concomitantly, to enhance lysosomal capacity in neurons and other cell types. Compounds which regulate such cellular functions will offset the accumulation of aberrant proteins found associated with various pathologies and, thus, provide a treatment to delay or slow neurodegenerative events including those underlying Alzheimer's disease, Parkinson's disease, and lysosomal storage disorders. Using this model, which displays pathogenic responses similar in sensitivity and temporal relationship to those found in Alzheimer's disease, compelling evidence has been found supporting a specific pathogenic cascade that is initiated by lysosomal dysfunction. The lysosomotropic conditions are followed by 1) modification of tubulin chemistries, 2) hyperphosphorylation and aggregation of the microtubule-associated protein tau, 3) concomitant destabilization of microtubules, 4) disruption of axonal and dendritic transport processes, 5) reduction in presynaptic components and, thus, maintenance, and 6) corresponding deterioration of postsynaptic structures and their electrical responses. This cascade likely leads to the types of synaptic deterioration linked to cognitive decline and dementia. Indeed, many of the pathogenic steps identified in the model were evident in correlative analyses using tissue samples from Alzheimer patients, the more prominent events being abnormal processing and accumulation of proteins and protein fragments.

To test if lysosomal capacity can be augmented to prevent abnormal protein processing, lysosomal modulators were identified that stimulate cellular feedback processes for the up-regulation of hydrolase levels. The modulators were applied to cultured rat brain slices at concentrations as low as 3 uM and were found to cause marked up-regulation of cathepsin D, the same hydrolase involved in the compensatory response in Alzheimer's disease. It was further determined that cathepsin D could be stably enhanced over a 20 day period without any reduction in synaptic markers. Oral administration of this modulator to rats at doses of 3 to 6 mg/kg also caused similar up-regulation of the cathepsin. More importantly, as the cathepsin hydrolase was modulated in rat brain slices over the extended period, hyperphosphorylated species of the microtubule-associated protein tau were gradually reduced in concentration. This suggests that lysosomal modulation dissipates pathogenic precursors of neurofibrillary tangles and prevents synaptic deterioration. Interestingly, a gradual increase in non-pathogenic tau isoforms also corresponded with the changes in cathepsin D and hyperphosphorylated tau levels. Thus, while purified cathepsin D has been shown to degrade tau proteins, lysosomal modulation may also influence phosphatases that convert pathogenic tau species into smaller non-pathogenic proteins. The normal tau molecules would then be more readily available to help stabilize microtubules and other transport machinery important for neuronal functions. Such a correlation between the two forms of tau was also evident in the hippocampus of aged mice, where the change vs. age relationships determined for hyperphosphorylated and non-phosphorylated tau were of equal but opposite slopes (Bahr and Vicente, 1998, *J. Neuropathol. Exp. Neurol.* 57:111-

121). Thus, along with the fact that lysosomal perturbations do not exhibit irreversible features (Bahr *et al.*, 1994, *Exp. Neurol.* 129:81-94), therapeutic strategies targeting lysosomal capacity appear not to be up against a "runaway train" syndrome. Lysosomal modulators may thereby intervene in the progression of certain types of neuronal atrophy, as well as in the cellular processes that contribute to synaptic loss and the severity of Alzheimers-type dementia.